

# Association of Tumor Necrosis Factor- $\alpha$ and Myeloperoxidase enzyme with Severe Asthma: A comparative study

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## Abstract

**Background:** Tumor necrosis factor-alpha (TNF- $\alpha$ ) may stimulate airway hyperresponsiveness in asthma, which is also affected by neutrophils activity. The latter can be determined indirectly by evaluating myeloperoxidase (MPO) activity. The insufficient studies that investigated the combined association of serum TNF- $\alpha$  and MPO with asthma was objective of this study.

**Methods:** A case-control study included 110-asthmatics besides 92-controls. All participants underwent venous sampling for TNF- $\alpha$  and MPO immunoassays. A percentage of predicted "forced expiratory volume in one second (FEV1%)", and the "peak expiratory flow rate (PEF/L)" of all participants were verified. The statistical analyses had done using SPSS V-25. The accuracy, specificity, sensitivity, and significance of both biomarkers to distinguish asthma examined "under the ROC-curves".

**Results:** High TNF- $\alpha$  levels observed among the controls( $p=0.006$ ), opposing the higher MPO levels among the patients( $p=0.00$ ). There were nonsignificant variations of two biomarkers between the treatment groups and nonsignificant correlations of MPO with FEV1 and PEF. There was a significant correlation of MPO with the TNF- $\alpha$  levels of all participants. The TNF- $\alpha$  showed lower sensitivity, specificity, and accuracy to diagnose asthma. There were no MPO differences according to asthma levels. The TNF- $\alpha$  was higher among the severe asthmatics significantly.

**Conclusions:** TNF- $\alpha$  may be a contributory particle for neutrophilic inflammation of severe asthma. MPO levels were significantly higher among asthmatics, whereas TNF- $\alpha$  levels were lower. TNF- $\alpha$  levels were higher among those with severe compared to mild/moderate asthma. The MPO level has a significant predictive capacity compared to TNF- $\alpha$  for distinguishing asthma from healthy subjects.

**Keywords:** Asthma, Inflammation, MPO, Neutrophils, TNF- $\alpha$ .

## Introduction

Asthma is one of the commonest airways inflammatory disorders presented with reversible bronchospasm, hyperresponsive and chronic inflammation of bronchial tree (1-4). Based on the kinds of inflammatory cells extant in the sputum, asthma was classified into four phenotypes: "eosinophilic, neutrophilic, mixed, and paucigranulocytic asthma" (5) Currently, few studies exposed

that each phenotype has a specific mechanism of action and responses to therapies. The precise subcellular mechanism that is involved in bronchial hyperresponsiveness is indistinct. Tumor necrosis factor-alpha (TNF- $\alpha$ ) is a cytokine produced in large amounts in asthmatic airways and involved in the progression of airways hyperresponsiveness. This is by direct change of the contractile

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features of the respiratory smooth muscle (5, 6). TNF- $\alpha$  may be a contributing molecule for both accumulation and activation of neutrophils in the airways of severe asthma (6). The activity of neutrophil cells could be determined indirectly by evaluating myeloperoxidase (MPO) enzyme activity (7, 8). Though still debatable, numerous observational studies have revealed reciprocal links between TNF- $\alpha$  and MPO with asthma (9-11). However, scarce scholars had investigated the combined association of both serum levels of TNF- $\alpha$  and MPO with asthma phenotypes.

**Objectives:** The study aims to evaluate the association of TNF- $\alpha$  and MPO together with asthma among Iraqi patients.

## **Materials and Methods**

### **Study Applicants**

The study was case control had included 110 asthma patients ( $41.9 \pm 9.1$  years old) besides 92 healthy controls ( $39.6 \pm 6.9$  years old). The asthmatic patients had cautiously selected from those attending the respiratory consultant clinics at Al-Imam Al-Sadiq hospital diagnosed as asthma (with no history of respiratory infections) based on the "Global Initiative for Asthma guidelines (GINA)" (12). The patients were divided into 2-sets (treated and untreated groups) depending on whether they were on regular or irregular (untreated) therapy for asthma. The recorded asthma therapy had involved inhaled/systemic steroids, oral/inhaled  $\beta$ -agonists, antileukotriene agents, and mixed inhalers.

### **Serological-detection of TNF- $\alpha$ and MPO**

Venous samples from all participants were collected, and the sera had stored at  $-20^{\circ}\text{C}$  till the time of TNF- $\alpha$  and MPO immunoassays. The serological assays had measured at the laboratories of the College of Pharmacy, University of Babylon, using an ELISA kit from "Human TNF- $\alpha$  (Tumor Necrosis Factor-Alpha) kit Elabscience® Biotechnology China". The MPO enzyme had assessed by MPO (Cloud-Clone Corp.®) ELISA kit USA.

### **Respiratory Function Test**

The percentage of the predicted "forced expiratory volume in one second (FEV1%)" as well as the "peak expiratory flow rate (PEF/L)" of all participants were verified with the existing spirometry at the hospital pulmonology unite "(Micro Medical® Spiro, USB, UK)".

### **Statistical Study**

The data distribution differences among the study groups had reached using SPSS V-25. Figures had shown as mean $\pm$ SD. Equally, Pearson's correlation had been finalized to estimate the statistical link. A student test had finished detecting characteristics among the treatment groups and group differences. The accuracy, specificity, sensitivity, and significance measurements of TNF- $\alpha$  and MPO to distinguish asthma had been examined "under the ROC curves".

### **Ethical Permission**

The study protocol had permitted by the health directorate and hospital ethical committee, and all candidates provided their informed consent. This work had done following "Helsinki-Statement".

## **Results**

The mean patients' ages were  $41.9 \pm 9.1$  compared to controls  $39.6 \pm 6.9$  years ( $p=0.12$ ). The males were dominant significantly in this study. The mean BMI ( $\text{kg}/\text{m}^2$ ) was parallel between the 2-groups, and the mean asthma duration among the patients was  $8.6 \pm 2.6$  years. The age revealed no significant link with spirometric values (results not shown). There were higher TNF- $\alpha$  levels observed among the healthy controls ( $p=0.006$ ), opposing the mean levels of MPO were high significantly among the patients ( $p=0.00$ ). Meanwhile, spirometric examination revealed a lower measure of FEV1% and PEF significantly among asthmatic patients (Table 1).

There was a nonsignificant variation of both MPO and TNF- $\alpha$  levels between the treated and untreated groups (Table 2).

The correlation of MPO with spirometric

variables and TNF- $\alpha$  had displayed in Table 3. It reveals a positive nonsignificant correlation of MPO with FEV1 and PEF and a significant correlation with the levels of TNF- $\alpha$  in the blood of all the studied participants.

The blood levels of MPO and TNF- $\alpha$  had been inspected in their predictive capability to distinguish asthma using the ROC curves statistics (Fig. 1 and Table 4). The MPO showed specificity (86.3), sensitivity (86.2),

and a significant accuracy rate of 84.6% (95%CI of 0.915-0.992). The TNF- $\alpha$  showed lower sensitivity and specificity (66.1 and 60.0) and a significantly lower accuracy rate 61.1% (95%CI of 0.929-1.0).

There were no significant differences in the distribution of MPO levels according to asthma levels. While the TNF- $\alpha$  was higher among the severe asthmatics significantly ( $p=0.036$ ) as revealed in Table 5.

**Table 1.** Main characteristics of the studied participants of the two groups.

	<b>Asthma patients (n=110) Mean<math>\pm</math>SD</b>	<b>Healthy controls (n= 92) Mean<math>\pm</math>SD</b>	<b>p-value</b>
<b>Age</b>	41.9 $\pm$ 9.1	39.6 $\pm$ 6.9	0.12
<b>Male sex (No/%)</b>	45/40.9	60/65.2	0.00
<b>BMI (kg/m<sup>2</sup>)</b>	28 $\pm$ 6.6	31.4 $\pm$ 8.4	NS
<b>Duration</b>	8.6 $\pm$ 2.6		
<b>Biomarkers</b>			
<b>TNF-<math>\alpha</math> (pg/ml)</b>	59.1 $\pm$ 91.4	123.2 $\pm$ 20. 9	0.006
<b>MPO (pg/ml)</b>	3222.5 $\pm$ 1280.8	1670.8 $\pm$ 991.6	0.00
<b>Pulmonary function tests</b>			
<b>FEV1</b>	77.6 $\pm$ 21.2	97.1 $\pm$ 10.3	0.00
<b>PEF</b>	65.6 $\pm$ 22.3	89.9 $\pm$ 18.8	0.00

**Table 2.** Distribution of myeloperoxidase and tumor necrosis factor- $\alpha$  between the study groups according to the treatment history.

	<b>Tumor necrosis factor-<math>\alpha</math></b>		<b>Myeloperoxidase</b>	
	Treated (n-67)	Untreated (n-43)	Treated (n-67)	Untreated (n-43)
<b>Mean<math>\pm</math>SD</b>	54.9 $\pm$ 104.4	55.7 $\pm$ 65.6	(3389.0 $\pm$ 1308.9)	(2928.8 $\pm$ 1191.9)
<b>p-value</b>	0.08		0.075	

**Table 3.** Correlation of myeloperoxidase with spirometric variables and tumor necrosis factor- $\alpha$ .

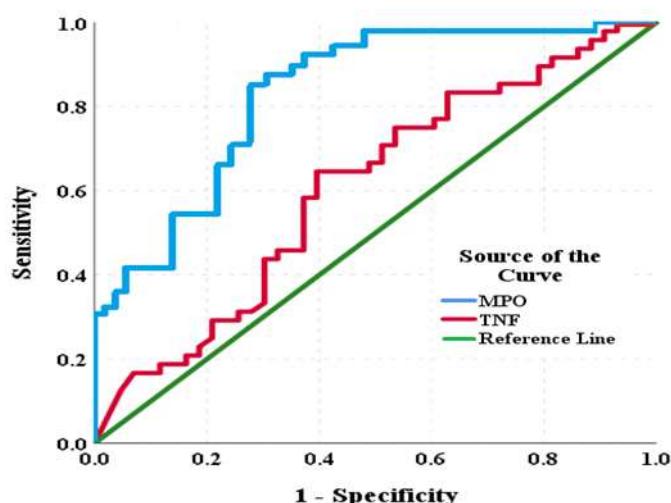
		<b>FEV1</b>	<b>PEF</b>	<b>MPO</b>
<b>MPO (pg/ml)</b>	Pearson Correlation	0.084	0.105	-
	Significance	0.4	0.312	-
<b>TNF-<math>\alpha</math> (pg/ml)</b>	Pearson Correlation	0.22	0.019	0.019
	Significance	0.002	0.89	0.8

**Table 4.** ROC curve analyses of TNF- $\alpha$  and MPO levels for prediction of Asthma.

<b>Biomarker</b>	<b>Sensitivity</b>	<b>Specificity</b>	<b>Accuracy</b>	<b>Significance</b>	<b>95% Confidence Interval</b>
Myeloperoxidase	86.2	86.3	0.846	0.000	0.789 - 0.903
Tumor necrosis factor- $\alpha$	66.1	60.0	0.611	0.01	0.528 - 0.694

**Table 5.** Distribution of myeloperoxidase and tumor necrosis factor- $\alpha$  in patients according to the severity of asthma.

	Asthma levels	Number	Mean $\pm$ SD	p-value
MPO	Mild	25	3147.9 $\pm$ 951.5	NS
	Moderate	35	2989.5 $\pm$ 1341.7	
	Severe	50	3509.8 $\pm$ 1397.4	
TNF- $\alpha$	Mild	25	23.6 $\pm$ 27.5	0.036
	Moderate	35	51.5 $\pm$ 81.7	
	Severe	50	73.5 $\pm$ 112.8	

**Fig. 1.** ROC-curves of the predictive ability of MPO and TNF- $\alpha$  to distinguish asthma.

## Discussion

In this paper, the discussion centers on the association of MPO and TNF- $\alpha$  with asthmatic patients. The data yielded by this study provides convincing evidence that the mean serum MPO levels were significantly higher among asthmatic patients compared to the healthy group, opposing TNF- $\alpha$  that revealed lower levels among asthmatic patients. The concentrations of TNF- $\alpha$  were high in severe asthma, compared to mild/moderate asthma. Levels of MPO have a significant predictive capacity compared to TNF- $\alpha$  for distinguishing asthma from healthy subjects.

The pathogenesis of asthma includes the expression of multifaceted inflammatory cyto-mediators. These cyto-mediators might perform pro-inflammatory, bronchospasm, or chemo-kinetic actions (13). The inflammatory response of air passages can be estimated by inspecting blood for cells or their mediators (14). The inflammatory role of neutrophils is

well-recognized, though its influence in asthma is still blurry. MPO is an enzymatic product of the primary neutrophilic granules (15). In line with the finding of our study, the underlying argument in favor of higher plasma (or sputum) MPO levels in asthma reflecting neutrophilic effects in severe asthma had been found by two researchers from Korea and Iraq (16, 17).

The mechanism behind the effects of TNF- $\alpha$  in asthma might be through several pathways. A direct influence of TNF- $\alpha$  on bronchial smooth muscle, the release of the "cysteinyl-leukotrienes LTC4 and LTD4", enhancing histaminic release from mast cells, chemoattracting for neutrophils and eosinophils, increasing the cytotoxic impact of eosinophils on airways endothelium, and intricate in the cytokine release from T-cells (18). TNF- $\alpha$  can enhance the expression of "intercellular adhesion molecule (ICAM) -1"

on and production of chemokines from respiratory endothelium and -in that way- can increase the neutrophil-based inflammatory response (19).

Though still debatable, several observational and clinical studies have revealed rather similar outcomes to our findings of levels of TNF- $\alpha$  in asthma. Kikuchi S. *et al.* from Japan reported that the TNF- $\alpha$  levels were not significantly different between asthma and healthy groups, and TNF- $\alpha$  was not associated with neutrophils in persistent severe asthma. Still, in sputum from severe asthma, the TNF- $\alpha$  levels were significantly associated with both the proportion of neutrophils and the MPO values (19). On the other hand, the mean plasma TNF- $\alpha$  levels in COPD and asthma-COPD overlap were significantly more than in bronchial asthma in a study that included 147 patients held last year in western Russia (20). The serum levels of TNF- $\alpha$  have not been raised in most asthma patients. Similarly, no significant difference in the levels between asthma and healthy groups in another study held in Iraq two years ago had been observed (10).

A growing body of evidence suggests a role for TNF- $\alpha$  in the mediation of some etiopathological mechanisms of asthma. In respiratory disease, TNF- $\alpha$  has a crucial role (though insufficient alone) in the initiation of hyperresponsiveness and the recruitment of pro and inflammatory cells in asthma. It had shown that TNF- $\alpha$  is elevated (though not significant) in sputum from peoples with neutrophilic, but not eosinophilic-asthma (21). Numerous clinical trials have measured TNF- $\alpha$  as a possible target for asthma and steroid-resistant inflammatory diseases (22). In a study of patients with moderate asthma, anti-TNF- $\alpha$  therapy was unable to alleviate pulmonary functions, though it did diminish exacerbations (23).

The fact that levels of TNF- $\alpha$  elevated in healthy subjects was not what we expected. However, lower levels of TNF- $\alpha$  amongst asthmatic patients compared to healthy controls generated by this study had been explained by several causes. A hereditary tendency to high TNF- $\alpha$  synthesis, predisposed

by "single nucleotide gene polymorphisms" (SNPs), may be significant. Firstly, significantly lower TNF- $\alpha$  values had detected from nasal smears associated with one or two SNPs of "TNF- $\alpha$ -308A alleles" (24). Extended use of steroid-inhalers reduced inflammation strikingly. Nevertheless, this lower inflammatory state had not always been linked with improved bronchial hyperresponsiveness (25, 26). Thirdly, even if TNF- $\alpha$  and interleukins were higher in the asthmatic airways, the interrelated impacts of these cytokines are hazy. IL-13 for example decreases inflammation and the synthesis of TNF- $\alpha$  from monocytes and alveolar macrophages (27, 28).

Interestingly, though indistinct reason, in this work a correlation between TNF- $\alpha$  and MPO had not been detected in the mild asthmatic. The cellular bases that provide TNF- $\alpha$  to the air-passages could be multicellular like respiratory macrophages or eosinophils, and epithelial cells, mast cells, and even neutrophils. Those with mild asthma may have good control with low-dose inhaled steroids, and hence it is likely that the production of inflammatory cytokines like TNF- $\alpha$  may be well repressed (29).

One of the potential intricate links correlating MPO, TNF- $\alpha$ , with asthma is transforming growth factor (TGF- $\beta$ ) and platelets-derived growth factor (PDGF). Epithelial cell injury is a significant element of asthma pathophysiology. TGF- $\beta$ 1 is a mediator having multicellular activities including apoptosis, injury (30-33), peribronchial fibrosis, and remodeling in asthma (33). PDGF is a powerful mitogenic product of different body cells (32, 34) acts as an immunomodulatory agent mediating bronchial remodeling also and a probable future therapeutic target (35).

A systematic review of methods applied to predict asthma exacerbation had shown that the contemporary models varied in policy, but surveys were consistent in missing strong proof (36). Further studies are essential regarding integrating a new biomarker in large representative peoples.

TNF- $\alpha$  may be a contributory particle for neutrophilic inflammation of patients with severe asthma. The mean serum MPO levels were significantly higher among asthmatic patients compared to the healthy group. TNF- $\alpha$  revealed lower levels among asthmatic patients. Levels of TNF- $\alpha$  were high among those with severe asthma compared to mild/moderate asthma. Levels of MPO have a significant predictive capacity compared to TNF- $\alpha$  for distinguishing asthma from healthy subjects.

We encountered some limitations in this study. A study in this limited number of

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patients had challenged a few limitations. Firstly, the coexistence of microbial infection cannot be excepted entirely. Secondly, the work had better include calculation of both neutrophils and eosinophils counts to discriminate asthma phenotypes (atopic and non-atopic). Thirdly, the study should comprise also the estimation of C-reactive protein.

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